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THE INNERVATION OF THE MUSCLE AND GLAND CELLS IN THE LIP OF THE SNAIL HELIX POMATIA L.

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Abstract

According to intensificated Co-labelling, both thick and thin nerve fibres innervate the muscle and gland cells, though the gland cells are predominantly innervated by thin fibres with small varicosities. The muscle cells are innervated by both serotonin and FMRFamide containing fibres with small varicosities. Among the gland cells immunoreactive fibres can scarcely be observed. The innervating fibres may partly originate from the cerebral ganglion since numerous cell bodies sending axonal processes to the lip became labelled after backfilling the lip branch of the medial lip nerve with Ni-lysine; and some of them proved to be serotonergic after 5,6-DHT treatment.
Key words: Helix pomatia, lip, innervation, muscle, gland

Introduction

It has been demonstrated that both central and peripheral neuronal elements take part in the regulation of the movements of the oral lobes during feeding behavior in *Helix pomatia* (KEMENES et al., 1982; HERNÁDI et al., 1984, 1987). In order to understand animal behaviour at the neuronal level, it is necessary to elucidate the neuronal connections at the peripheral level in effector organs as well as in the central nervous system (CNS). In the *Helix* lip, which is an effector organ taking part in feeding movements (SCHULTZ, 1938; KIECKEBUSH, 1953; KEMENES et al., 1985), the musculature and glandular mass are targets of both the central and peripheral nervous systems. The aim of this study was to investigate the innervation of muscle and gland cells by using Cobalt labelling of the innervating neuronal fibres. Furthermore, pigment induction and light microscopical immunocytochemistry were used to characterise the transmitter or modulator content of the innervating neuronal fibres by applying 5,6-DHT as well as serotonin (5HT) and FMRFamide antibodies.

Materials and methods

Adult specimens of the snail *Helix pomatia* were used for the experiments. The ganglion complex (CNS) and the lip with the medial lip nerve were excised and fixed on silgar with small needles. The lip branch of the medial lip nerve was cut, and the distal end was put into an open vaselin cup containing aqueous solution of Cobaltic-lysine (200 μM). Thereafter the cup was enclosed with vaseline and the

preparation was covered with ringer solution. After 48h exposure at 4 °C, the Co ions in the lip were precipitated in a cold phosphate buffer (pH 7.4) saturated with H_2S gas. The lip was dehydrated and embedded in paraffin, and 20 μm thick serial sections were cut. After deparaffination the serial sections were subjected to intensification procedure (GÖRCS et al., 1979; HERNÁDI et al., 1987) to visualize the Co-sulfide in the nerve fibres. After intensification the serial sections were dehydrated and covered with canadabalsam and studied with a light microscope.

In order to determine the localization and the serotonergic nature of the neurons sending axonal processes to the lip, 5,6-dihydroxytryptamine neurotoxine was injected into the body cavity of each animal in a dose of 10 mg/kg body weight which induces the pigmentation of the serotonergic neurons (S-RÓZSA et al., 1986; HERNÁDI et al., 1988, 1989). Thereafter the CNS was excised and Nickel-lysine backfilling was done through the proximal end of the lip branch of the medial lip nerve that was put into a vaselin cup containing 200 μM Ni-lysine. After 48h transporting time the Ni ions were visualized with rubeanic acid giving a bluish colour (QUICK and BRACE, 1979; HERNÁDI et al., 1984) to the neurons that send axonal processes to the lip. The serotonergic neurons sending axons into the lip had bluish colorization and contained rusty-brown pigment granules in their somata. The CNSs were dehydrated, embedded into canadabalsam and studied as whole mount preparations. The map of the Ni — labelled blue and the double labelled (blue and pigmented) serotonergic neurons was drawn from microscope by using a drawing apparatus.

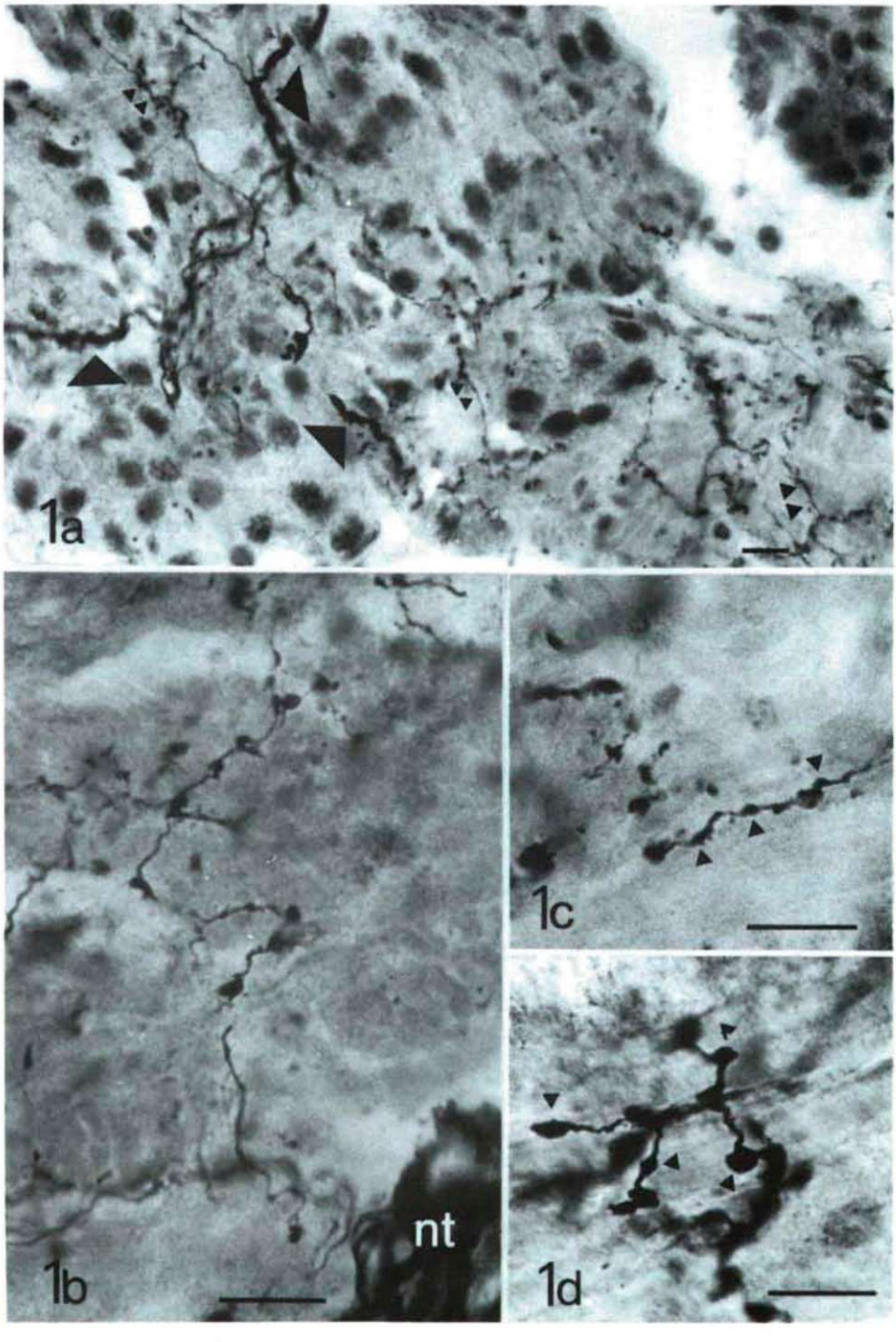
For light microscopical immunocytochemistry the lips were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 12h at 4 °C and were washed overnight in the buffer. The samples were dehydrated and embedded in paraffin. Immunocytochemical procedure was performed on 15 μm thick deparaffinated sections according to Sternberger (1979). Both the serotonin and FMRFamide antibodies were used in 1:3000 dilution in phosphate buffered saline containing 0.3% Triton X—100 (PBSTX). Antiserum specificity for 5HT and FMRFamide was tested by replacing the primary antibody with normal rabbit serum at the same dilution. After the development of the immunocytochemical reaction with DAB-H_2O_2 the sections were dehydrated in ethanol followed by xylene and covered with canadabalsam.

Results

LIGHT MICROSCOPICAL ANALYSIS OF THE INTENSIFICATED SERIAL SECTIONS

In the serial sections a dense labelled fibre system can be observed (Fig. 1.a). The majority of the labelled fibres can be observed in the musculature, while in the glandular mass intermingled with muscle cells only a few groups of labelled fibres can be detected running parallel with the gland cells (Fig. 2.a). The labelled fibres that originate from large labelled nerve trunk (Fig. 1.b) run over the muscle cells have different diamaters and numerous varicosities (Fig. 1.a). The varicosities are either small or large on the labelled fibres (Fig. 1.c, d). Among the gland cells nerve fibres with small varicosities are dominant (Fig. 2.b) while fibres with large varicosities can only scarcely be observed (Fig. 2.c).

Fig. 1. In the intensificated section of the lip a rich arborization of the innervating fibres can be seen in the musculature, consisting of thin (small triangles) and thick (large triangles) nerve fibres (Fig. 1.a). The solitary fibres originate from large labelled nerve trunks (nt) (Fig. 1.b). The labelled fibres have small (Fig. 1.c) or large (Fig. 1.d) varicosities (small triangles) over the muscle cells. scale bars: 10 μm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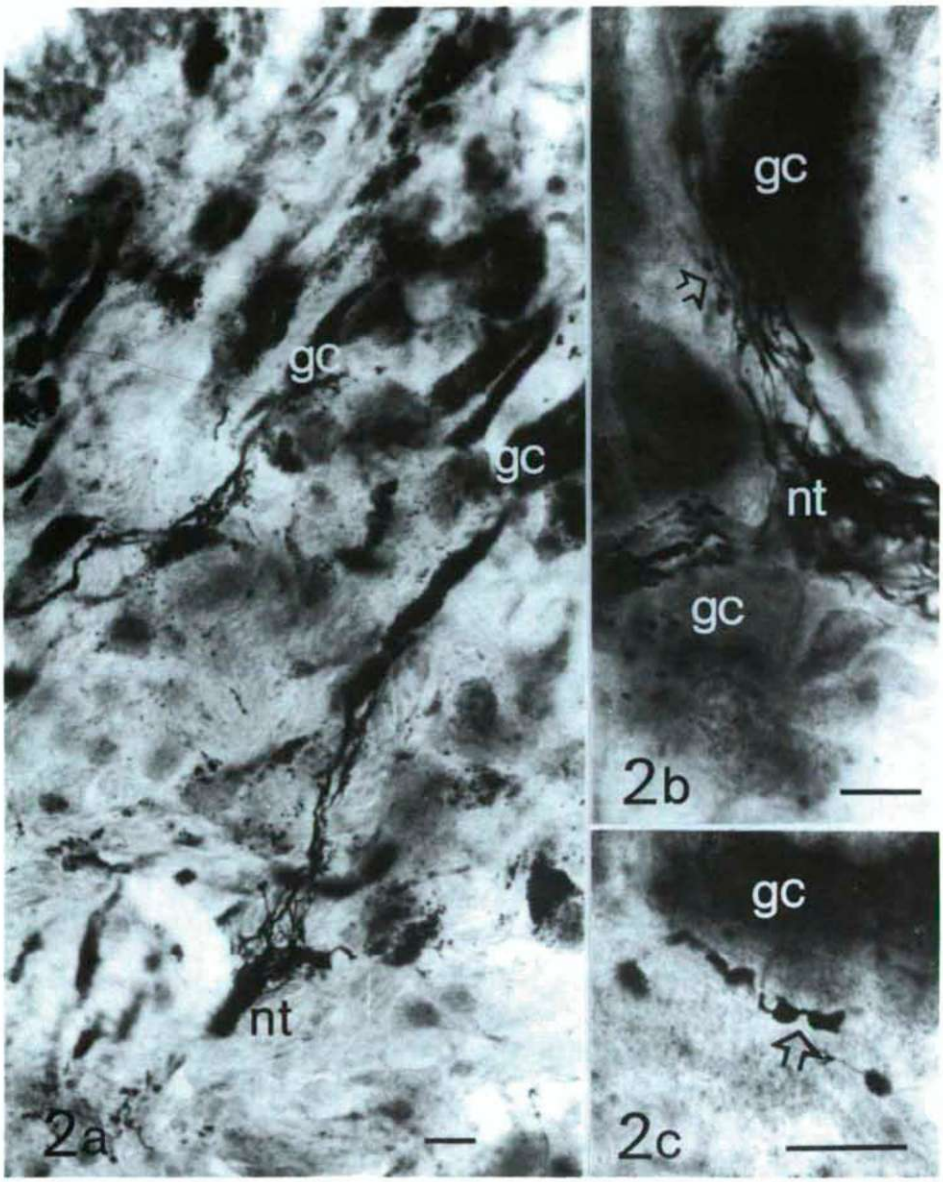


Fig. 2. Among the gland cells (gc) small trunks of labelled fibres (nt) run parallel with the gland cells (Fig. 2.a). The labelled fibres invaginate into the gland cells and have small (Fig. 2.b) and large (Fig. 2.c) varicosities on them (open arrows). scale bars: 10 μm

FMRFAMIDE AND SEROTONIN (5HT) IMMUNOREACTIVE ELEMENTS IN THE LIP

FMRFamide immunoreactive fibres were frequently detected in the musculature of the lip, but only scarcely among the gland cells (Fig. 3.). Immunoreactive fibres are observable in nerve trunks and also as solitary fibres with small varicosities among the muscle cells (Fig. 3.). Immunoreactive nerve cell bodies cannot be detected.

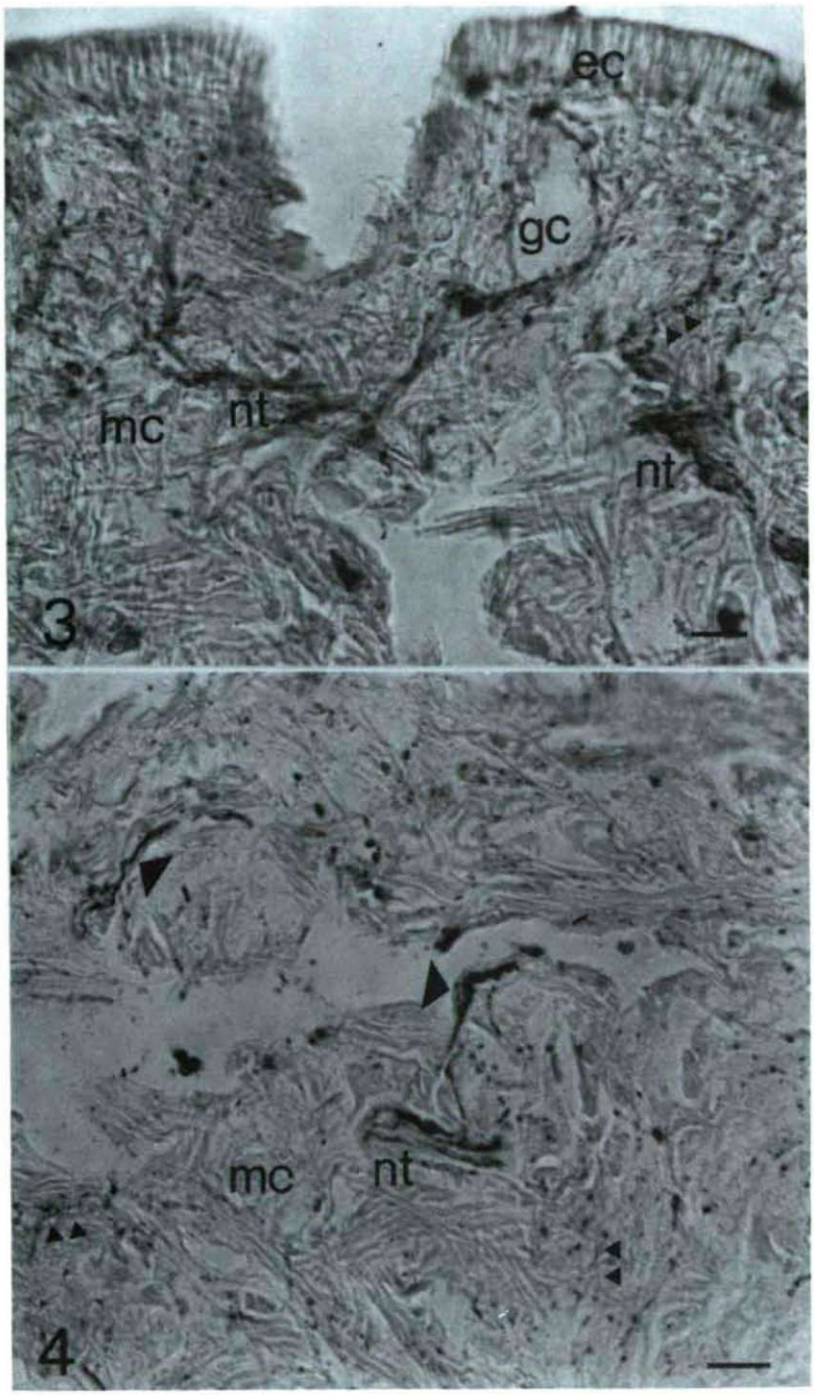
5HT-immunoreactive fibres can be seen all over the musculature of the lip but rarely seen among the gland cells (Fig. 4.). The immunoreactive fibres run in groups in the nerve trunk or as thin and thick solitary fibres among the muscle cells. Numerous varicosities can be observed on single immunoreactive fibres. Immunoreactive neuronal somata cannot be seen in the sections.

MAPPING OF CEREBRAL NEURONS THAT SEND AXONAL PROCESSES TO THE LIP

In the cerebral ganglion the neuronal cell bodies that send axonal processes to the lip can be seen as blue cells after Ni-lysine backfilling of the nerve branch of the medial lip nerve. Blue labelled neurons can be detected in all parts of the cerebral ganglion, but they are dominant on the ventral surface (Fig. 5.). The serotonergic neurons appear as rusty-brown pigmented cell bodies after 5,6-DHT injection. They are located only on the ventral surface of the cerebral ganglion (detailed description see: S-RÓZSA et al., 1986; HERNÁDI et al., 1988, 1989). After Ni-lysine backfilling 12—15 pigmented serotonergic neurons become blue coloured in the metacerebrum around the metacerebral giant cell (MGC) (Fig. 5.).

Discussion

In the lip the organization of the muscle cells into longitudinal and transversal fibres is similar to that described in the body wall of other gastropod species (ROGERS, 1968, 1969; PLESCH, 1977). According to the analysis of the intensificated Co-labelled fibres, the lip musculature and glandular mass are innervated by numerous thin and thick labelled fibres with small and large varicosities, which demonstrate that they are innervated by two morphological types of fibres. The gland cells are innervated predominantly by thin fibres with small varicosities, while the muscle cells are innervated by a roughly equal number of both thin and thick fibres. On the basis of the Co-lysine backfilling of the lip as well as the Ni-lysine backfilling of the cerebral ganglion, the innervating fibres may originate partly from the labelled neurons located in the lip and partly from the labelled neurons in the cerebral ganglion that send neural processes to the lip through the medial lip nerve. According to the light microscopical immunocytochemistry both 5-HT and FMRFamide immunoreactive fibres can be seen in the lip. The distribution of the 5-HT and FMRFamide immunoreactive fibres is similar; they are dominant among the muscle cells and have small varicosities. Among the gland cells intermingled with muscle cells these fibres can scarcely be observed. The morphological appearance of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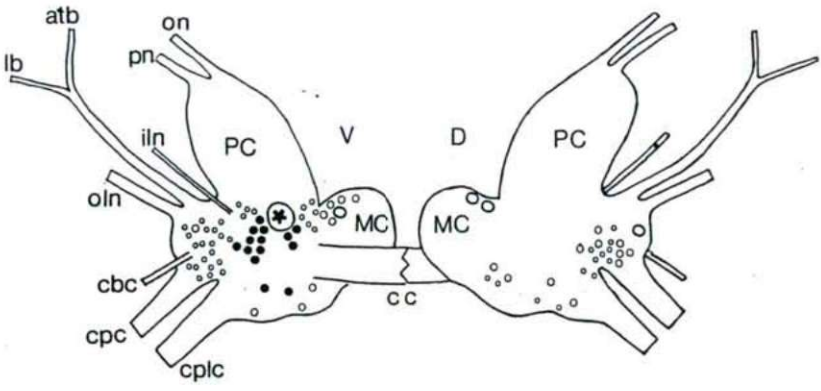

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Fig. 5. The distribution of Ni-labelled cerebral neurons sending axonal processes to the lip on the surface of the cerebral ganglion after, 5,6-DHT pigmentinduction. dark simbols: nickel-labelled pigmented serotonergic neurons, open simbols: nickel-labelled non pigmented neurons, cbc: cerebro-buccal connective, cc: cerebral comissure, cplc: cerebro-pleural connective, cpdc: cerebro-pedal connective, iln: inner labial nerve, mln: medial labial nerve, oln: outer labial nerve, on: olfactory nerve, pn: penis nerve, lb: lip branch of the medial lip nerve, tb: anterior tentacular branch of the medial lip nerve, PC: procerebrum, MC: mesocerebrum, asterisk: Metacerebral giant cell. scale bar: 10 μm

the 5-HT and FMRFamide immunoreactive fibres are very similar to the thin Co-labelled fibres with small varicosities. Therefore, it is difficult to establish whether they contain both 5-HT and FMRFamide in the same fibre or whether they represent separate morphological types of innervating fibres. The 5-HT and FMRFamide immunoreactive fibres may originate from the cerebral ganglion since neither 5-HT nor FMRFamide immunoreactive cell bodies were observed in the lip, but numerous Ni-labelled bluish neurons proved to be serotonergic in the cerebral ganglion sending axonal processes to the lip demonstrating the dominancy of the CNS in the serotonergic and FMRFamidergic innervation.

Fig. 3. FMRFamide immunoreactive fibres (Fig. 3.) can be frequently detected in the paraffin section of the lip. The thin labelled fibres are branching from nerve trunks (nt) and have small varicosities (triangles) among the muscle cells (mc) but not among the gland cells (gc). Nerve cell bodies can not be observed in the sections. ec: epithelial cell layer; scale bar: 10 μm

Fig. 4. Serotonin immunoreactive fibres can be observed predominantly among the muscle cells (mc). The labelled fibres have thick (large triangles) and thin appearances with varicosities (small triangles). nt: nerve trunk. scale bar: 10 μm

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